INSECT CELLS AND THEIR POTENTIAL AS STABILIZATION BARRIERS FOR DNA OF MULTIPLE AND SINGLE NUCLEOPOLYHEDROVIRUSES AGAINST ULTRAVIOLET-B–SIMULATED SUNLIGHT INACTIVATION

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SUMMARY

A cell line from *Trichoplusia ni* (TN-CL1) infected with the *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV-HPP) and a cell line from *Helicoverpa zea* (BCIRL-HZ-AM1) infected with the *Helicoverpa zea* single nucleopolyhedrovirus (HzSNPV/BrCL2) were subjected to ultraviolet-B (UV-B) irradiation at a predetermined level of exposure that would inactivate greater than 95% of the virus suspended in the liquid. The working hypothesis was that the homologous insect cells would utilize their inherent deoxyribonucleic acid (DNA) repair mechanism(s) to prevent, repair, or at least mitigate the damaging effects of UV-B light on viral DNA synthesis. We attempted to determine this by using infected cells that were subjected to UV-B irradiation at different postinoculation periods under two experimental conditions of exposure: (1) shielded, and (2) nonshielded. Of the two cell lines infected with their respective homologous viruses, the virus from TN-CL1 cells was the least sensitive to UV-B light because the extracellular virus (ECV) and occlusion body (OB) levels of virus-infected TN-CL1 cells were higher than those of the virus-infected BCIRL-HZ-AM1 cells. Production of ECV and OB from both cell lines was lower in the exposed, nonshielded treatment than in the exposed, shielded treatment. However, AcMNPV-HPP was produced in enough quantity to indicate that TN-CL1 might impart a level of protection to the virus against UV light.

Key words: UV-B irradiation; baculovirus inactivation; insect cells.

INTRODUCTION

Insect cells are known to be highly resistant to short-wavelength solar ultraviolet (UV) irradiation, specifically at a 254-nm wavelength (UV-C) of the electromagnetic spectrum. Evidence for this comes from several studies that have demonstrated that cultured insect cells have the ability to show considerable resistance to the 254-nm UV irradiation relative to other cultured nonarthropod cells as measured by F_0, which is the fluence required to reduce survival by e^{-1} on the exponential portion of a cell survival curve (i.e., to 37% of its previous value). For example, the lepidopteran cell line TN-368 was seven times more resistant to the lethal effects of UV-C (250–280 nm) than were the V-79 Chinese hamster cells (Koval et al., 1977). Similarly, in a later study investigating the role of photoreactivation and liquid-holding recovery, TN-368 cells were found to be 12 times more resistant than V-79 cells to UV-C irradiation (Koval, 1986). It has also been established that TN-368 cells have a higher resistance to UV-C irradiation than do rat kangaroo cells (Wade and Trosko, 1983), chick-embryo cells (Bronk et al., 1984), fish cells (Mano et al., 1980), frog cells (Griggs and Bender, 1978; Rosenstein and Setlow, 1980), and several prokaryotes (Harm, 1980; O'Brien and Houghton, 1982). Similarly, the *Drosophila melanogaster* cell lines, WR69-DM-1 (Schneider's line 1) and WR69-DM-2 (Schneider's line 2), derived from embryos, showed higher resistance to UV-C irradiation than did nonarthropod organisms (Trosko and Wilder, 1972; Koval, 1987). However, variation in the level of resistance to UV irradiation also exists between insect cell lines derived from different orders. For example, in a study by Koval (1987) to investigate the extent of involvement of the photoreactivation mechanism in the repair of deoxyribonucleic acid (DNA) damaged by UV light, the resistance by the *Drosophila* WR69-DM-2 cell line F_0 at 21 J/m² was found to be four times lower than the F_0 determined for TN-368 cells (Koval, 1986).

Specific types of UV-B damage to DNA have been identified in mammalian cells, such as the Chinese hamster V-79 cells, and include DNA single-strand breaks and DNA–protein cross-links (Matsumoto et al., 1991), the formation of cyclobutane pyrimidine dimers (5–6) and pyrimidine–pyrimidone (6–4) photoproducts (Ellison and Childs, 1981; Okaichi et al., 1989), and base pair substitution events (Colella et al., 1986). The induction of some of these DNA lesions by shorter-wavelength UV light (254 nm) has also been demonstrated in two cell lines from the Indian meal moth, *Plodia interpunctella*, namely IAL-PID2 (Styer et al., 1989) and UMN-PIE1181 (Styer and Griffiths, 1992) and in TN-368 cells (Koval, 1986), and two *D. melanogaster* cell lines (Trosko and Wilder, 1972; Koval, 1987). Cellular mechanisms available for the repair of (5-6)

DNA adducts can proceed via monomerization of the (5-6) dimers during photoreactivation, whereas the (6-4) lesions are unaffected by enzymatic photoreactivation. Similar responses by insect cells might be expected on exposure to UV-B light, but no data are yet available.

Natural UV-sunlight is known to be an important environmental factor responsible for the inactivation or reduced infectivity of baculoviruses in the field. Investigations have shown that the effects of UV sunlight on the stability and persistence of baculoviruses may lead to a reduction in their potential effectiveness as biological control agents (David et al., 1968; Bullock et al., 1970; Broome et al., 1974; Ignoffo and Hostetter, 1977). However, nothing has been reported in the literature concerning the relationship between baculovirus stability after exposure to UV light (when it is contained within the insect cell), in terms of the de novo viral DNA replication and the maintenance of the virus integrity.

In this study we investigated the capability of the insect cells to serve as a barrier to prevent or at least mitigate the damaging effects of UV-B light on intracellular virus replication, subsequent occlusion body (OB) formation, and release of extracellular virus (ECV) from the host cell.

MATERIALS AND METHODS

Insect cell lines and medium. TN-CL1 cells, a clone (McIntosh and Rechtoris, 1974) produced from the TN-368 cell line derived from ovarian tissues of the cabbage looper, *Trichoplusia ni* (Hink, 1970), and BCIRL-HZ-AM1 cells derived from pupal ovaries of the corn earworm, *Helicoverpa zea* (McIntosh and Ignoffo, 1981), were used in this study. The two cell lines were grown as monolayer cultures in ExCell® 401 (JRH Biosciences, Lenexa, KS) supplemented with 10% inactivated (65° C and 30 min) fetal bovine serum (FBS) (Invitrogen, Purchase, NY), 50 μg/ml streptomycin, and 50 units/ml penicillin (Sigma Chemical Co., St. Louis, MO). Both cell lines were previously identified by DNA amplification fingerprinting–polymerase chain reaction (McIntosh et al., 1996).

Virus. Extracellular virus of AcMNPV-HPP, a clone of AcMNPV (McIntosh et al., 1985), and HzSNPV/BrCL2, a clone of HzSNPV (McIntosh and Ignoffo, 1981), were used to infect cells at a multiplicity of infection (MOI) of 1.0.

UV-B source and cell line exposure technique. A UV-Stratalinker 2400 (Stratagene, La Jolla, CA), with a battery of 5 UV (312 nm) 15-W bulbs and an internal sensor to monitor the level of UV radiation, was used to irradiate cells under dark conditions. Cells in a Costar 12-well plate (Corning Costar, Corning, NY) were placed at a distance of 14 cm from the radiation source and exposed for 3 h, which is equivalent to receiving an incident dose (fluence) of 23.9 kJ/m². Based on preliminary studies using AcMNPV-HPP and HzSNPV/BrCL2, each individually in 2 ml of the media in a Costar 12-well plate, a 3-h exposure period resulted in greater than 95% inactivation of the ECV population, as analyzed by the tissue culture infectivity dose ($TCID_{50}$) titration. Consequently, the 3-h period was selected for subsequent UV-B exposure experiments. Temperature within the UV chamber rose gradually from an initial 28° C to a high of 39° C after 1.5-h into the test and was stable at this temperature for the remaining 1.5 h exposure. If there is any detrimental effect of high temperature on the cells and the virus, it would be reflected in low virus production in the UV-shielded (virus + cells) exposed treatment because this was also intended to factor out the effect of UV light exposure, while concurrently still showing the possible temperature effect. The estimated UV-B$^{312 nm}$ half-life of AcMNPV-HPP ECV under the above experimental conditions was 114 min, and for HzSNPV/BrCL2 ECV the estimated UV-B$^{312 nm}$ half-life was 24 min. In all experiments of this study, the lid to the Costar 12-well plate was placed during exposure of the cells to UV radiation because there was no evidence in the previous experiments to demonstrate a significant difference between the effects of UV exposure on insect cells with or without the Costar lids (unpublished data). TN-CL1 cells were seeded at 1×10^5 cells/ml in 2 ml of the culture media in each of three wells of a Costar 12-well plate and allowed to attach for 1 h. Cells were inoculated with AcMNPV-HPP at an MOI of 1.0 for 2 h on a Bellco rocker platform (Bellco Technology, NJ) at a setting of 2.5 at room temperature.

After the inoculation period, cells in each well were washed twice with 2 ml of ExCell 401 + 10% FBS to remove residual inoculum, and 2 ml of fresh medium was added. One plate with infected cells was covered with two sheets of aluminum foil (shielded), whereas the other plate was not covered with aluminum foil (nonshielded), and both were exposed to UV for 3 h at the following postinoculation time periods: 8, 12, 18, and 24 h. To determine a baseline of infectivity for each virus, cells were seeded at the earlier mentioned concentration, infected at an MOI of 1.0 with no UV exposure, and incubated at 28° C. Two other plates covered with an aluminum foil and uncovered and containing only the virus in the medium were also prepared for each time period to evaluate the effect of UV on the virus inoculum alone. After the UV exposure periods, cells were incubated at 28° C until the 72 h postinoculation period, when ECV and OB were recovered and quantitated. The titer of the supernatant collected after 72 h postinoculation that contained ECV was determined using TN-CL1 cells as an indicator cell line, according to a previously described method (McIntosh et al., 1985). Dilution–end point assays, based on the number of positive wells that contained OB, were performed by employing the indicator cell line in duplicate for each of the replicates, and results were recorded after 7 d of incubation at 28° C. The number of OB produced in each replicate was enumerated with a hemacytometer. Essentially, the same procedure outlined previously, to determine ECV and OB production under the various UV-B exposure periods for TN-CL1 cells, was also used for BCIRL-HZ-AM1 cells. The only difference was that the number of BCIRL-HZ-AM1 cells seeded in each well of a Costar 12-well plate was 5×10^4 cells/ml instead of 1×10^5 cells/ml, and the supernatant containing HzSNPV/BrCL2 ECV was titrated using BCIRL-Hz-AM1 cells.

Determination of cell proliferation in the presence and absence of UV-B light. To determine the normal level of DNA synthesis or cell proliferation under typical growth conditions without virus but in the presence and absence of UV-B light, we used an immunocytological assay kit to measure the incorporation of 5-bromo-2′-deoxy-uridine (BrdU) into TN-CL1 and BCIRL-Hz-AM1 DNAs during cell replication (Roche Molecular Biochemicals, Indianapolis, IN). In general, the assay was followed as specified by the manufacturer but with several minor modifications. TN-CL1 and BCIRL-Hz-AM1 cells were seeded at 1×10^5 cells/ml in 2 ml of ExCell® 401 + 10% FBS and contained antibiotics, as described previously. Cells were seeded in triplicate wells of a 12-well Costar plate. Three treatments were set up for each cell line: (1) cells exposed to UV light for 3 h (nonshielded); (2) cells exposed to UV light for 3 h (shielded); and (3) cells not exposed to UV light (control). After exposure, cells were allowed to acclimate for 1 h at room temperature. The BrdU labeling reagent was diluted 1:1000 with the growth medium to give a final concentration of 10 μmol BrdU/L and added to each well for 3 h at 36° C under the three treatment regimes described earlier. The labeling reagent was then removed, and cells were washed three times with 1000 μl of the washing buffer (provided in the kit), fixed with 500 μl of 70% ethanol (in 50 m*M* glycine buffer, pH 2.0) for at least 20 min at −20° C, and then incubated for 30 min at 36° C in 200 μl of anti-BrdU diluted 1:10 with the incubation buffer No. 3. The anti-BrdU reagent was removed, and the cells were washed three times with 1000 μl of the washing buffer. Cells were covered with 200 μl of anti-mouse Ig–alkaline phosphatase (diluted 1:10 with PBS [Sigma No. 4417]) for 30 min at 36° C and were then washed three times with the washing buffer. Cells were examined under a light microscope after 30 min of exposure to a colored substrate solution containing 13 μl nitroblue tetrazolium salt in 70% dimethylformamide (NBT) and 10 μl X-phosphate solution in 3 ml substrate buffer. The level of BrdU incorporation into cells was measured by counting the number of cells stained with the BrdU label within three randomly selected microscopic fields for each of the three replicate wells using an IM 35 inverted microscope (Carl Zeiss, Thornwood, NY).

Statistical analysis of the data. AcMNPV-HPP ECV and HzSNPV/BrCL2 OB data were analyzed, after passing both normality and equal variance tests, using a two-factor analysis of variance (ANOVA) followed by a Tukey's multiple comparison procedure to determine significant differences in all pairwise mean comparisons. However, AcMNPV-HPP OB and BCIRL-HZ-AM1 ECV data were analyzed, after both untransformed and transformed data failed the normality and equal variance tests, using Kruskal-Wallis ANOVA on ranks followed by the Dunn's pairwise multiple comparison procedure. The percentage data from the BrdU labeling study were arcsine–square root transformed and analyzed using a one-factor ANOVA to determine the differences between the three test treatments.

TABLE 1

MEAN VALUES[a] OF AcMNPV-HPP ECV[b] PRODUCTION IN TN-CL1 CELLS EXPOSED TO UV[312 nm] LIGHT FOR 3 H AT VARIOUS POSTINOCULATION PERIODS (UNDER VARIOUS EXPERIMENTAL CONDITIONS)[c,d]

Postinoculation period (h)	Nonshielded (cells + virus)	Shielded (cells + virus)	Nonshielded (virus only)	Shielded (virus only)	Virus activity unexposed to UV (virus + cells)
8	12.73 ± 1.05	23.04 ± 6.41	0.0022 ± 0.0003	0.024 ± 0.003	16.48 ± 3.44
12	12.48 ± 3.06	26.05 ± 7.76	0.003 ± 0.0006	0.07 ± 0.008	6.59 ± 1.60
18	18.32 ± 2.59	29.20 ± 5.36	0.022 ± 0.007	0.079 ± 0.014	13.70 ± 2.98
24	6.84 ± 1.97	17.65 ± 3.96	0.0068 ± 0.002	0.29 ± 0.53	14.29 ± 4.49

[a] $\bar{x}$ ± SEM.
[b] ECV titer values = $TCID_{50}$/ml × 10^6.
[c] ECV was collected at 72 h postinoculation; multiplicity of infection = 1.0; UV-shielded virus only (*column 5*) was included to check the functioning of the UV chamber; UV-shielded (cells + virus) (*column 3*) and UV-shielded virus only (*column 5*) were also both used as controls for temperature in the UV chamber.
[d] UV, ultraviolet; ECV, extracellular virus.

TABLE 2

MEAN VALUES[A] OF HzSNPV/BrCL2 ECV[b] PRODUCTION IN BCIRL-HZ-AM1 CELLS EXPOSED TO UV[312 nm] LIGHT FOR 3 H AT VARIOUS POSTINOCULATION PERIODS (UNDER VARIOUS EXPERIMENTAL CONDITIONS)[c,d]

Postinoculation period (h)	Nonshielded (cells + virus)	Shielded (cells + virus)	Nonshielded (virus only)	Shielded (virus only)	Virus activity unexposed (virus + cells)
8	0.44 ± 0.28	15.13 ± 0.21	4.98 ± 0.87	12.48 ± 1.77	9.00 ± 0.99
12	5.78 ± 0.40	7.11 ± 0.89	0.01 ± 0.00	0.24 ± 0.099	4.21 ± 0.58
18	0.01 ± 0.00[e]	13.05 ± 0.00	0.049 ± 0.004	1.27 ± 0.33	2.22 ± 0.90
24	0.23 ± 0.11	29.06 ± 0.94	0.32 ± 0.05	3.70 ± 1.17	10.98 ± 2.52

[a] $\bar{x}$ ± SEM.
[b] ECV titer values = $TCID_{50}$/ml × 10^4.
[c] ECV was collected at 72 h postinoculation; multiplicity of infection = 1.0.
[d] UV, ultraviolet; ECV, extracellular virus.
[e] Replicate values were all ≤10^3, and so a quantity of 0.01 was selected to represent the actual mean value.

RESULTS

Effect of UV-B irradiation on ECV production. TN-CL1 cells infected with AcMNPV-HPP for 18 h postinoculation and then subjected to UV-B irradiation under shielded conditions showed the highest mean ECV production followed by the 12-h UV-shielded treatment and the 8-h UV-shielded treatment (Table 1). When TN-CL1 cells infected with AcMNPV-HPP were exposed to UV-B under shielded conditions, there was no significant difference in the ECV titers, regardless of the postinoculation period (Table 1; $P = 0.166$, among the different postinoculation periods; $P = 0.453$ in the interaction between time and treatment). However, ECV production between UV-B treatments was significantly higher in the shielded treatment than in the nonshielded treatment ($P < 0.001$). In the UV-B shielded experiments, when BCIRL-Hz-AM1 cells infected with HzSNPV/BrCL2 were exposed to UV-B, cells infected before UV-B exposure for 24 h showed the highest mean ECV production followed by the 8-h and the 18-h postinoculation treatments (Table 2). Although there were no significant differences in the BCIRL-HZ-AM1 ECV production in the pairwise between mean treatment comparisons of shielded treatment across all postinoculation periods, ECV production by cells in the 18-h nonshielded treatment was significantly lower than that in the nonshielded 8-, 12-, and 24-h treatments ($P < 0.05$). ECV produced in nonshielded infected cells before each of the exposure time periods should be inactivated by UV-B light once each exposure time period begins.

This is evident based on our treatment data, which showed that ECV in the growth medium (no cells present) is readily inactivated by UV-B.

Consequently, whatever ECV is produced by the cells after inoculation up until the virus is collected at 72 h should be a reflection of the actual effect of UV-B exposure on the cells and their ability to support virus production.

Effect of UV-B irradiation on OB production. Shielded TN-CL1 cells infected with AcMNPV-HPP and UV-B irradiated at 18 h postinoculation resulted in the highest mean OB production followed by the 12-h shielded treatment and the 12-h nonshielded treatment (Table 3). Furthermore, none of the pairwise between mean treatment comparisons showed any statistically significant differences, except between the 18-h UV-shielded treatment and the 8-h UV-exposed (nonshielded) treatment ($P < 0.05$). BCIRL-Hz-AM1 cells infected with HzSNPV/BrCL2 for 12 h and then subjected to UV irradiation showed the highest mean OB production (Table 4). There were statistically significant differences in OB production among all the postinoculation periods within both the nonshielded and shielded treatments ($P = 0.007$).

Cell DNA synthesis in the presence and absence of UV-B light. Compared with nonexposed infected cells, uninfected exposed TN-CL1 cells showed no significant difference in the number of cells undergoing DNA synthesis, as measured by the incorporation of the BrdU label among the three experimental conditions in this study

TABLE 3

MEAN VALUES[a] OF AcMNPV-HPP OB[b] PRODUCTION IN TN-CL1 CELLS EXPOSED TO UV[312 nm] LIGHT FOR 3 H AT VARIOUS POSTINOCULATION PERIODS (UNDER VARIOUS EXPERIMENTAL CONDITIONS)[c,d]

Postinocula-tion period (h)	Nonshielded (cells + virus)	Shielded (cells + virus)	Virus activity unexposed (virus + cells)
8	1.25 ± 0.06	2.04 ± 0.17	2.13 ± 0.09
12	4.50 ± 0.32	5.00 ± 0.03	2.69 ± 0.02
18	3.92 ± 0.73	5.81 ± 0.35	2.75 ± 0.19
24	1.95 ± 0.075	2.96 ± 0.28	2.60 ± 0.06

[a] $\bar{x} \pm$ SEM; *note: columns 3 and 5 of Table 1 were also the controls for data described in this table.*

[b] OB values = OB $\times 10^6$ OB/ml.

[c] OB were collected at 72 h postinoculation; multiplicity of infection = 1.0.

[d] UV, ultraviolet; OB, occlusion body.

TABLE 4

MEAN VALUES[a] of HzSNPV/BrCL2 OB[b] PRODUCTION IN BCIRL-HZ-AM1 CELLS EXPOSED TO UV[312 nm] LIGHT FOR 3 H AT VARIOUS POSTINOCULATION PERIODS (UNDER VARIOUS EXPERIMENTAL CONDITIONS)[c,d]

Postinocula-tion period (h)	Nonshielded (cells + virus)	Shielded (cells + virus)	Virus activity unexposed (virus + cells)
8	0.99 ± 0.17	5.58 ± 0.68	2.13 ± 0.09
12	5.78 ± 0.40	7.11 ± 0.89	7.20 ± 2.91
18	0.1 ± 0.00^e	4.00 ± 0.59	6.63 ± 0.71
24	4.40 ± 0.58	5.31 ± 0.45	8.23 ± 0.86

[a] $\bar{x} \pm$ SEM.

[b] OB values = OB $\times 10^5$ OB/ml; *note: columns 3 and 5 of Table 2 were also the controls for data described in this table.*

[c] OB were collected at 72 h postinoculation; multiplicity of infection = 1.0.

[d] UV, ultraviolet; OB, occlusion body.

[e] Replicate values were all $\leq 10^4$, and so a quantity of 0.1 was selected to represent the actual mean value.

($P > 0.329$). Mean values of $47.8 \pm 0.036\%$ in the nonexposed cells, $41.5 \pm 0.033\%$ in the nonshielded, exposed cells, and $39.3 \pm 0.045\%$ in the control UV-exposed (shielded) cells underwent active DNA synthesis. It appears that UV-B light exposure does not seem to have a detrimental effect on TN-CL1 DNA synthesis. However, in the case of BCIRL-Hz-AM1 cells, in the nonshielded treatment relative to the shielded treatment, the percentage of cells incorporating BrdU was significantly reduced ($P < 0.05$) ($24.7 \pm 0.022\%$ in nonshielded versus $34.4 \pm 0.032\%$ in the shielded), whereas differences in cell DNA synthesis between the nonexposed and shielded treatment ($34.7 \pm 0.028\%$), and the nonexposed and nonshielded treatment were not significantly different ($P < 0.05$).

DISCUSSION

The results of the present investigation demonstrate that insect cells infected with a baculovirus may provide some protection to baculoviruses against exposure to high doses of UV-B. Whether the protective effect is because of a physical barrier or a repair mechanism afforded by the host cell needs further elucidation.

In this study, we investigated the interaction of incident UV-B irradiation on insect cells infected with baculoviruses. Our working hypothesis was that homologous insect cells might either act as a physical barrier (at the cellular membrane level) blocking damaging UV light or employ their various inherent cellular DNA repair mechanisms to repair both viral DNA as well as their own DNA damaged by UV-B light. Grounded on a comparative study of AcMNPV-HPP and HzSNPV/BrCL2 OB and ECV production in TN-CL1 and BCIRL-Hz-AM1 cells, respectively, we have attempted to determine indirectly whether or not host insect cells have the potential capability to protect the intracellular virus when exposed to incident UV-B light at various postinoculation periods.

Infected TN-CL1 and BCIRL-HZ-AM1 cells responded in a similar manner to exposure to UV light in the unprotected state (nonshielded) compared with their shielded counterparts by producing lower levels of both ECV and OB, regardless of the postinoculation time of exposure. However, despite the significantly lower ECV titers of AcMNPV-HPP produced in nonshielded infected TN-CL1 cells relative to the infected TN-CL1 cells that were shielded, AcMNPV-HPP still produced titers ranging from 12×10^6 TCID$_{50}$/ml at the 8- and 12-h postinoculation periods to 18×10^6 TCID$_{50}$/ml at the 18-h postinoculation period. Why there is a decrease in the ECV titer of the cells when exposed to UV-B at 24 h is unknown. Perhaps the majority of the ECV produced by the cells by 24 h postinoculation had already been released into the medium and were inactivated by UV. In contrast, infected BCIRL-HZ-AM1 cells (nonshielded) responded differentially when exposed to UV light, except at the 12-h postinoculation period, relative to shielded cells by producing between 12- and 1300-fold less ECV titer depending on the postinoculation exposure period. There was also a differential response between the two cell lines in the percentage of uninfected cells undergoing DNA synthesis during exposure to UV-B light as measured by the incorporation of the BrdU label. This differential response of the two cell lines to UV-B irradiation when in an uninfected state might indicate the potential of how well the two cell lines can respond to UV exposure in terms of the production of their homologous viral ECV and OB. Uninfected BCIRL-HZ-AM1 cells appeared to be more sensitive than uninfected TN-CL1 cells when exposed to UV-B by having a lower percentage of cells actively undergoing DNA synthesis. In vitro studies have shown that there is a similar phenomena of differential sensitivity of uninfected cells to UV irradiation in other lepidopteran cell lines, as well as in cells derived from different insect orders (Koval, 1983; Styer and Griffiths, 1992). Differences in the DNA synthesis between the two cell lines on UV-B irradiation when in an uninfected state appears somewhat suggestive of how well the individual infected cell lines will protect an enclosed virus against UV light. This is demonstrated by the generally higher ECV titers at the 8- and 18-h postinoculation exposures. Counts of OBs were also higher, ranging from 1.25×10^6 OB/ml at an 8-h postinoculation exposure to 4.5×10^6 OB/ml at a 12-h postinoculation exposure, in TN-CL1 cells.

OB production was also affected by the manner in which cells were treated, with nonshielded cells producing a lower number of OBs than shielded cells, when exposed to UV light. The generally lower titers in all the controls (virus + cells; nonexposed) relative to the UV-shielded (cells + virus) in both single nucleopolyhedrovirus and multiple nucleopolyhedrovirus might be a result of lower incubation temperatures ($28°$ C in the incubator versus $37°$ C in the UV chamber).

We have shown in this study that the ECV and OB productions of a single nucleopolyhedrovirus and a multiple nucleopolyhedrovirus in their respective permissive cell lines were lower in the exposed, nonshielded treatment than in the exposed, shielded treatment. However, AcMNPV-HPP was produced in enough quantity to indicate that TN-CL1 might impart a level of protection to the virus against UV-B light.

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